REVIEW

Role of Non-esterified Fatty Acids in the Pathogenesis of Type 2 Diabetes Mellitus

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Over 30 years after the original description of Randle's cycle, the mechanisms by which elevated circulating non-esterified fatty acids might be causally related to glucose intolerance in Type 2 diabetes mellitus remain uncertain. This review examines the evidence that elevated plasma NEFA can inhibit glucose-stimulated insulin secretion by pancreatic beta-cells and impair glucose- and insulin-stimulated glucose disposal by peripheral tissues. Experimental evidence beginning to examine the mechanisms of these phenomena is discussed. © **1998 John Wiley & Sons, Ltd.**

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Introduction

More than 30 years ago, Randle *et al*.[1] proposed that the glucose/fatty acid cycle might cause the alterations in insulin action observed in non-insulin-dependent (Type 2) diabetes mellitus. They documented that elevations in non-esterified fatty acid (NEFA) concentrations increase lipid oxidation and decrease the activities of pyruvate dehydrogenase and phosphofructokinase.[2] The resulting accumulation of glucose 6-phosphate, in turn, inhibits hexokinase, glucose uptake, and glucose oxidation. More recently, acute increases in plasma NEFA concentration were shown to decrease insulin-mediated glucose uptake[3,4], and acute decreases in NEFA concentration induced by antilipolytic agents were accompanied by an increase in insulin-mediated glucose uptake.[5] Enhanced NEFA availability and lipid oxidation may inhibit non-oxidative glucose metabolism as well as glucose oxidation.[6] The inhibitory effect of NEFA on non-oxidative glucose metabolism may result from the inhibition of glycogen synthase.[6]

Despite the demonstrated negative impact of plasma NEFA on insulin-mediated glucose uptake, the role of NEFA as a risk factor for the development of Type 2 diabetes has been examined only recently. In a prospective study of the development of Type 2 diabetes, 190 Pima Indians were investigated.[7] Body composition was determined by underwater weighing, waist-to-thigh circumference was used as the index of body fat distribution, and subcutaneous abdominal adipose tissue cells were obtained for *in vitro* studies. Oral glucose tolerance tests (75 g) and the euglycaemic hyperinsulinaemic glucose clamp (insulin infusion rate: 40 mU $m^{-2}h^{-1}$) were used to measure changes in glucose tolerance and insulin-stimulated glucose uptake. In a subset of 103 participants, the plasma insulin response to intravenous glucose (25 g injected over 3.6 min) also was determined. Mean duration of follow-up was 4.0 ± 2.4 years; during this period, 47 subjects developed Type 2 diabetes. Of the *in vitro* measures, only average adipose cell volume (risk ratio = 2.4; 95 % CI = 1.2–4.8) was a significant risk factor for the development of Type 2 diabetes; basal or isoprotenerol-stimulated lipolysis and median effective dose (ED_{50}) for insulin suppression of isoproterenol-stimulated lipolysis were not.

Of the *in vivo* measures, elevated plasma NEFA concentration was predictive of the development of Type 2 diabetes (risk ratio = 3.5; 95 % CI = 2.0–6.1) even after adjusting for gender, per cent body fat, and insulin-mediated glucose uptake (Figure 1), and of body fat distribution and fasting plasma triglyceride concentration (risk ratio = 2.3; 95 % CI = 1.1–4.7). However, when acute insulin response (AIR) was added to the model including gender, per cent body fat, and insulin-mediated glucose uptake, high plasma NEFA concentration was no longer predictive of Type 2 diabetes (risk ratio = 1.0; 95 % CI = 0.5–2.1) (Figure 1). Plasma triglyceride concentration (risk ratio = 1.5; 95 % CI = 0.7–3.4) and basal lipid oxidation (risk ratio = 1.0; 95 % CI = 0.5–2.1) were not predictive of development of Type 2 diabetes when adjusted for age, gender, per cent body fat and body fat distribution.

Abbreviations: AIR acute insulin response, GSIS glucose-stimulated insulin secretion, NEFA non-esterified fatty acids

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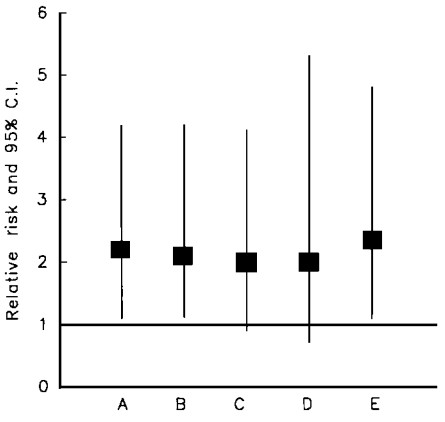

The predictive role of FFA was independent of:

A: Age, sex, body fat (%), waist/thigh ratio
B: Sex, body fat (%), insulin–mediated glucose uptake
C: Sex, body fat (%), insulin–mediated glucose uptake fat cell size
D: Sex, body fat (%), insulin–mediated glucose uptake, acute insulin response
E: Sex, body fat (%), waist/thigh ratio, insulin–mediated glucose uptake, fasting plasma triglycerides concentration

Figure 1. Relative risk and 95 % confidence intervals (CI) for the predictive role of FFA on the development of Type 2 DM. All models were calculated in 190 subjects (except D, 103 subjects). Data are a graphic representation of Table 3 of Paolisso *et al.*[7] with the authors' permission

These results have been confirmed in 4057 Caucasians participating in the Paris Prospective Study.[8] In this study, a 0.12 mM increase in fasting plasma NEFA concentration over a follow-up period of 2 years was associated with a 30 % increase in risk of deterioration of glucose tolerance. The effect of high plasma NEFA concentration was independent of central obesity, plasma glucose, and insulin concentrations. Further, when the participants were categorized according to tertiles of fasting plasma NEFA concentration at baseline, more than half of those with impaired glucose tolerance (62 %) were in the upper quartile. The rate of deterioration from normal to impaired glucose tolerance paralleled the increase in tertiles of fasting plasma NEFA concentration. Unfortunately, the Paris Prospective Study was not designed to assess changes in insulin-mediated glucose uptake and insulin secretion by direct, specific measurements. Thus, conclusions regarding the impact of NEFA on insulin sensitivity and secretion in the development of Type 2 diabetes in Caucasians remain to be confirmed.

Nevertheless, the above data clearly indicate that elevated plasma NEFA concentration is a risk factor in the development of Type 2 diabetes. Why and how plasma NEFA concentrations contribute to the deterioration of glucose tolerance is less clear. Elevated NEFA concentrations may profoundly inhibit insulin-mediated glucose uptake. Evidence that the predictive effect of NEFA on the development of Type 2 diabetes is independent of insulin-mediated glucose uptake, however, suggests that other pathways must be considered, and an inhibitory effect of elevated NEFA upon glucose-stimulated insulin secretion (GSIS) cannot be ruled out. In the study in Pima Indians, the predictive effect of NEFA on the development of Type 2 diabetes was no longer significant when AIR was added to the multivariate analysis. Since these data were reported, a growing body of evidence has accumulated showing that elevated NEFA may inhibit GSIS. The following sections review the possible mechanisms of the negative effects of NEFA on both insulin secretion and action.

Evidence for a Negative Impact of NEFA on Insulin Secretion

β-Cell defects are thought to be involved in the pathogenesis of Type 2 diabetes associated with obesity.[9] The progression from obesity to Type 2 diabetes can be induced by a high-saturated-fat, high-sucrose diet in susceptible rodents[10] and is often associated with a high-fat diet in susceptible humans.[11] Under these dietary stimuli, insulin secretion increases to meet the need to store both glucose and the excess fat. In obesity, there is hypersecretion of insulin. However, in Type 2 diabetes, despite sometimes elevated basal insulin secretion, the main islet defect is the inability of glucose to stimulate adequate secretion and a reduction in the potentiating action of glucose on neurohormonal agonists.[12] One recently suggested mechanism for these β-cell anomalies is that they occur in genetically susceptible individuals who exhibit greater sensitivity to conditions that elevate long-chain acyl-CoA, possibly because of inappropriate expression or activity of metabolic enzymes, signal transducing effectors, or ionic channels.[9]

Thus, the primary question is whether environmental factors may contribute to β-cell insensitivity to glucose. Several studies have demonstrated that elevated NEFA concentration may interfere with insulin secretion through a process designated by Unger as lipotoxicity.[13] It has been proposed that in obesity, elevated plasma NEFA is caused by insensitivity of adipocytes to the antilipolytic action of insulin,[13] by the increase in adipocyte mass, by incomplete suppression by feeding,[14] or by an overexpression of TNF-α. In islets of normal rats, increased NEFA concentration has been shown to stimulate basal insulin secretion, induce β-cell proliferation, and enhance glucose metabolism, which increases basal insulin secretion and potentiates insulin responses to all stimuli.[13] Because fatty acid concentrations in tissues are proportional to plasma levels of NEFA, insulin action and insulin secretion are balanced, and glucose tolerance remains normal. After rats become diabetic, GSIS is absent and β-cell GLUT2 is reduced.

Hyperglycaemia does not appear to be the metabolic cause of glucose 'blindness' or of GLUT2 loss in β-cells.[15] Elevated NEFA concentration was postulated to produce an inhibitory effect on GSIS,[16] glucokinase,[17] phosphofructokinase,[18] glucose oxidation,[13] and glucose-stimulated insulin biosynthesis.[13] This hypothesis is

consistent with Unger's data in obese Zucker prediabetic rats showing that elevations in NEFA precede the development of diabetes, and that levels of NEFA are more elevated in prediabetic than in non-prediabetic rats.[13] An inhibitory effect of NEFA on insulin secretion has been demonstrated by Sako and Grill[19] who observed a 50 % reduction in GSIS by the perfused pancreas of normal rats after 48-h infusion of a triglyceride emulsion, and by Elks[16] who noted a more substantial loss of GSIS in perfused islets exposed to 1 mmol l^{-1} palmitate. How NEFA impair GSIS is not clear. NEFA may inhibit the action of glucose via operation of the Randle cycle within β-cells. Zhou and Grill[20] showed that exposure of islets to fatty acids *in vitro* led to profound inhibitions of GSIS. The inhibitory effect of NEFA on GSIS required a long induction time (6–24 h) and was not due to β-cell damage; it was reversible, abolished by another nutrient secretagogue, and rapidly reversed by etomoxir.[20] A 48-h culture with palmitate or oleate significantly decreased oxidation of D-(U-^{14}C) glucose and this inhibition was reversed by etomoxir,[20] an irreversible inhibitor of carnitine palmitoyltransferase I (CPT1). These latter data are particularly important because they show that the inhibitory effects of NEFA on GSIS and biosynthesis are exerted at the level of glucose oxidation. These authors also found that production of ^{14}CO from D-(3, 4-^{14}C) glucose, which reflects the decarboxylation of pyruvate due to pyruvate dehydrogenase activity, was depressed after long-term exposure to NEFA.

NEFA may also alter the expression of a number of gene-encoding enzymes of intermediary metabolism.[9] Dietary NEFA, particularly if polyunsaturated, suppress the mRNA abundance and the expression of fatty acid synthase and acetyl-CoA carboxylase in rat liver and in adipose tissue. It has also been demonstrated that long-chain NEFA markedly affect the expression of genes coding for enzymes of intermediary metabolism in β-cells. Long-term exposure of the clonal pancreatic β-cells line INS-1 to NEFA decreased acetyl-CoA carboxylase mRNA and protein at low glucose and antagonized the inductive effect of sugar.[21] In contrast, NEFA induced the liver type CPT-1 transcript in INS-1 cells.[21] Thus, it has been suggested that alterations in the expression of metabolic enzymes by NEFA may account for the β-cell insensitivity to glucose or for alterations in insulin secretion following long-term exposure of β-cells to elevated FFA concentrations.[22] This hypothesis is attractive, because acetyl-CoA carboxylase and CPT-1 may act as nutrient sensors and because the metabolism of all classes of insulin secretagogues converge towards these enzymes. Peroxisome proliferator-activated receptors (PPARs), members of the steroid/thyroid/retinoid hormone receptor family, are also implicated in the process of β-cell insensitivity.[9,23] PPARs mediate the induction by certain long-chain NEFA of a number of gene-encoding proteins involved in NEFA oxidation and biosynthesis as well as ketogenesis.[24,25] Thus, PPARs, like malonyl-CoA, are thought to be key factors in the interactions among nutrients, metabolism, and genes in the context of the substrate competition between glucose and NEFA.[26]

The results of the above *in vitro* studies have been confirmed *in vivo*. Twelve healthy participants underwent a 24-h Intralipid (10 % triglyceride emulsion, Intralipid, Pharmacia, Milan Italy) infusion at a rate of 0.4 ml min^{-1} and a simultaneous infusion of heparin (a bolus of 200 U followed by 0.2 U min^{-1} per kg body weight) to raise plasma NEFA concentration two- to threefold.[27] At baseline, at 6 h and at the end of the 24-h period, an intravenous glucose bolus (25 g in 1 min) was administered to assess AIR. Finally, an intravenous glucose bolus was performed after a 24-h wash-out period to study the recovery process.

Compared to baseline AIR, short-term (6-h) Intralipid infusion was associated with a significant increase in AIR, but long-term (24-h) Intralipid delivery was associated with an inhibition of AIR (Figure 2). After the 24-h wash-out period, the β-cell response to glucose was comparable to baseline. Further, the changes in AIR and NEFA concentration were significantly correlated at 6 h and at 24 h (Figure 3). These data indicated that a short-term increase in plasma NEFA concentration has a stimulatory effect on GSIS, but a long-term increase in plasma NEFA has an inhibitory effect. These effects were not seen when the stimulus to insulin secretion was hyperglycaemic clamp.[28] Under those conditions, the pancreas was continuously stimulated by a high plasma glucose concentration (±9.0 mmol l^{-1}), which can stimulate overactivity of the glycolytic pathway. This overactivity might be strong enough to offset the inhibitory role of NEFA on GSIS. This hypothesis has been tested in a small sample (n = 4) of healthy non-obese individuals who underwent three different tests in the presence or absence of a 24-h 10 % Intralipid infusion (0.4 ml min^{-1}): an isoglycaemic glucose clamp (mean plasma glucose concentration: 5 mM); a hyperglycaemic glucose clamp (mean plasma glucose concentration: 7 mM); or a hyperglycaemic glucose clamp (mean plasma glucose concentration 9 mM).

Each test was separated from the other for at least 3 days; insulin secretion rate was calculated using the method of Polonski *et al*.[29] Preliminary results showed that Intralipid infusion increased plasma NEFA concentrations from 0.21 ± 0.07 mM to approximately 1.51 ± 0.23 mM without significant differences among other experimental conditions; these values are similar to those in the previous study.[27] Elevated plasma NEFA concentration significantly inhibited the insulin secretion rate at 7 mM glucose but not at baseline or 9 mM glucose (Figure 4). These results paralleled the changes in respiratory quotient.

The evidence that elevated NEFA concentration inhibits GSIS is strengthened by the association of reduced plasma NEFA concentration with increased GSIS. Several compounds with antilipolytic activity have been reported to be effective in reducing hyperglycaemia,[13] but their effects on islet function have not been characterized.

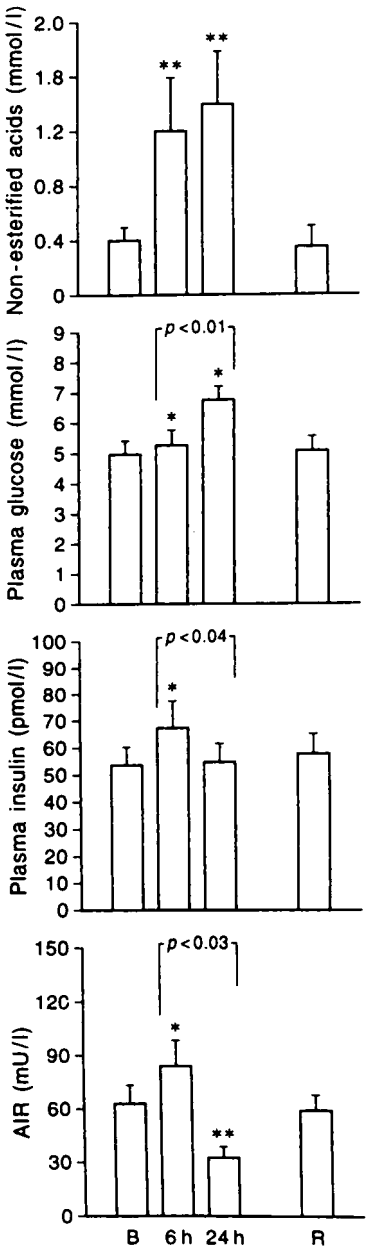

Figure 2. Changes in plasma NEFA, glucose, and insulin concentration, and acute insulin response (AIR) during intralipid infusion in healthy subjects (n = 12) at baseline (B), after 6 h and after 24 h intralipid infusion; R = recovery. All results are means ± SD. Statistically significant differences vs baseline were: *$p<0.01$; **$p<0.001$. Reproduced with the permission of the authors (Paolisso *et al.*[27]) and copyright holder

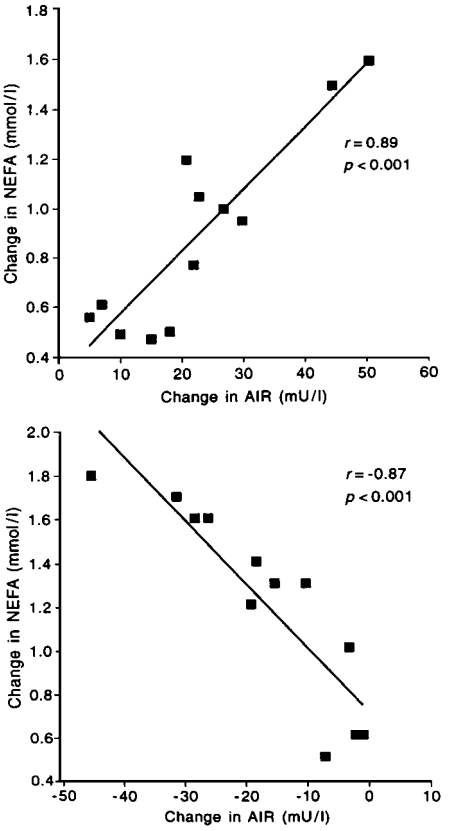

Figure 3. Simple correlations between changes in fasting plasma NEFA and changes in acute insulin response (AIR) after 6 h (top) and 24-h (bottom) Intralipid infusion in healthy subjects (n = 12). Reproduced with the permission of the authors (Paolisso *et al.*[27]) and copyright holder

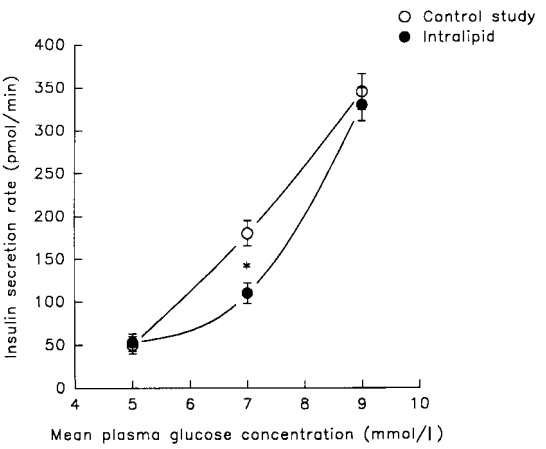

Figure 4. Dose–effect curve between insulin secretion rate and mean plasma glucose concentration along an hyperglycaemic glucose clamp in absence and presence of 24 h 10 %. Intralipid infusion in healthy subjects (n = 4); *$p<0.05$

We have recently investigated the effect of Acipimox in first-degree relatives of individuals with Type 2 diabetes,[30] subjects at high risk for development of Type 2 diabetes with low insulin-mediated glucose uptake,[31] and β-cell response to glucose.[32] Acipimox administration lowered plasma NEFA concentrations and that this reduction was associated with an increase in GSIS.

It has also been reported that NEFA induced suppression of insulin output might be mediated by nitric oxide (NO).[33] In fact, when normal islets were cultured in 2 mM NEFA, NO production and basal insulin secretion increased slightly. In cultured prediabetic Zucker diabetic fatty islets, NEFA induced a fourfold greater rise in NO, upregulated mRNA of inducible nitric oxide synthase (iNOS), and reduced insulin secretion.[33] In contrast, nicotinamide and aminoguanidine, which lower NO, prevented the NEFA-mediated increase in iNOS mRNA,

reduced NO, and minimized the loss of insulin secretion.[33]

In summary, the results of both *in vitro* and *in vivo* studies appear to demonstrate that elevated NEFA concentrations inhibit glucose-stimulated insulin secretion. However, the *in vivo* inhibitory effect of elevated NEFA concentration on β-cell responses to glucose occurs only when plasma glucose concentrations are not high enough to offset the increased NEFA oxidation via the Randle cycle.

Evidence for a Negative Impact of NEFA on Insulin Action

The inhibitory role of NEFA on insulin action is much more clearly defined and has been more extensively studied.[34] There is no doubt that elevated NEFA concentrations impair glucose oxidation in the muscle through the Randle cycle.[1] Several[4,35,36] but not all[37,38] studies also demonstrate that elevated NEFA inhibits insulin-mediated glucose uptake in healthy individuals as well as in those with Type 2 diabetes. Recently, it has been demonstrated that elevated plasma NEFA concentration inhibits glucose oxidation (within 1 to 2 h) and later inhibits insulin-mediated glucose uptake (generally after 4 h).[34,39] Further, there is strong evidence that physiological elevations of plasma NEFA lower peripheral insulin sensitivity in a dose-dependent fashion.[34] With regard to the effect of NEFA on the liver, the data are consistent with a stimulatory effect of NEFA upon gluconeogenesis.[34,40,41] The proposed mechanisms include increased production of adenosine 5′-triphosphate (ATP) and nicotinamide adenine dinucleotide (NADH) and activation of pyruvate carboxylase by acetyl-CoA generated via fatty acid oxidation. Rebrin *et al*[42] have recently reported that in conscious dogs a relationship exists between plasma NEFA and hepatic glucose output at steady state and during dynamic insulin changes. However, lowering plasma NEFA with nicotinic acid or Acipimox has been variably reported to decrease,[43,44] increase,[45] or not affect[46] hepatic glucose output. When plasma NEFA were raised during a euglycaemic or hyperglycaemic-hyperinsulinaemic glucose clamp, insulin suppression of hepatic glucose output was only partially inhibited.[34] Thus, the available human data demonstrate that elevated NEFA concentrations inhibit insulin-mediated glucose uptake in skeletal muscle while they stimulate gluconeogenesis in the liver.

In considering the pathways involved in NEFA-mediated inhibition of insulin-mediated glucose uptake, at least two mechanisms can be hypotheszed: inhibition of glucose transport or phosphorylation at an early stage (within 2 to 3 h) or inhibition of muscle glycogen synthase activity at a later stage (after 4 h). The cellular and molecular mechanisms responsible for these transport/phosphorylation and glycogen defects are unknown. Possibilities include NEFA-induced inhibition of insulin-mediated glucose uptake via accumulation of glucosamine pathway metabolites, or an NEFA-related interference with GLUT4 gene expression in skeletal muscle and adipose cells.[34] *In vitro*[47] and *in vivo*[48] elevated NEFA concentrations are associated with a rise in plasma free radical concentrations. Interestingly, the inhibitory effect of NEFA on insulin-mediated glucose uptake was partially counterbalanced by a simultaneous infusion of reduced glutathione, a known potent antioxidant. These data are consistent with previous results showing that oxidative stress may be a further cause of insulin resistance.[49] Finally, elevated plasma NEFA concentrations might induce changes in membrane fluidity.[50,51] Insulin receptors are embedded in the lipid bilayer of plasma membranes, and there is evidence to suggest that altering the fatty acid content of membranes can alter insulin receptor accessibility, insulin binding, and insulin action.[51]

Conclusions

The role of elevated NEFA in the development of Type 2 diabetes is strongly supported by cross-sectional and longitudinal data. Less clear are the pathophysiological mechanisms responsible for the negative effect of NEFA on glucose metabolism. After plasma NEFA concentrations are elevated (due to insulin resistance at the adipose tissue level, to increased adipose tissue mass, or to elevated plasma TNF-α factor) there is increased NEFA flux to peripheral tissues. This is associated with increased utilization of NEFA as an energy source. High NEFA oxidation contributes to impaired insulin-mediated glucose uptake, is responsible for lipotoxicity of the β-cells with a secondary impairment of glucose-stimulated insulin secretion, and activates gluconeogenesis in the liver (Figure 5). In addition, changes in plasma free radical concentrations and in membrane fluidity might contribute to the negative impact of NEFA on insulin-mediated glucose uptake. However, despite the growing body of evidence describing roles of plasma NEFA at various levels, the molecular mechanisms linking elevated plasma NEFA concentration to impaired insulin-mediated glucose uptake and glucose-stimulated insulin secretion need further investigation.

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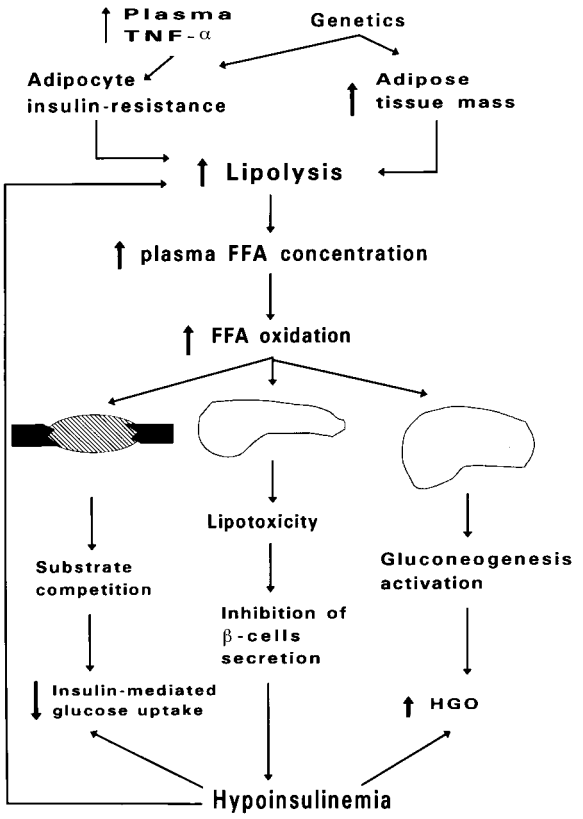

Figure 5. Mechanisms explaining the role of plasma non-esterified fatty acids (NEFA) concentration on the development of Type 2 diabetes mellitus

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